DEVELOPMENT, GROWTH, AND EGG PRODUCTION OF *CENTROPAGES ABDOMINALIS* AND *NEOCALANUS FLEMINGERI* FROM THE EASTERN SUBARCTIC PACIFIC

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A

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ABSTRACT

Copepods dominate oceanic mesozooplankton in terms of abundance and biomass thus contributing a significant source of secondary production. I determined development, growth, and egg production of *Centropages abdominalis* and *Neocalanus flemingeri* at temperatures representative of the northern Gulf of Alaska in spring. Median development times from eggs to adults were 42 and 59 days for *C. abdominalis* at 5 and 7°C, respectively, and 117 days from eggs to copepodite stage five for *N. flemingeri* at 5°C. Average copepodite growth rates were 0.08 and 0.17 d^{-1} for *C. abdominalis* at 5 and 7°C, respectively, and 0.15 d^{-1} for *N. flemingeri* at 5°C. *In situ* egg production of *C. abdominalis* was 37 ± 22 eggs $female^{-1}$ d^{-1} (mean ± S.D.), corresponding to a growth rate of 0.14 d^{-1}. Lifetime fecundity of *N. flemingeri* determined at 5°C was 535 ± 258 eggs $female^{-1}$ (mean ± S.D.). Comparing these results to *in situ* populations reveals that *C. abdominalis* may be food limited during the summer and fall, while *N. flemingeri* is likely not food limited in late spring. Overall, this information helps clarify the life history patterns of these two species and allows production to be estimated and models of secondary production created for conditions within the Gulf of Alaska.

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INTRODUCTION

The three orders of copepods, Calanoida, Cyclopoida, and Harpacticoida, each have more than 1,000 species represented both in freshwater and marine systems (Raymont, 1983). The two species addressed in this thesis, *Centropages abdominalis* and *Neocalanus flemingeri*, belong to the order Calanoida, which are holoplanktonic, mostly marine, and have development stages which proceed from eggs through six naupliar stages and six copepodite stages. Copepods are a vital component of oceanic communities and comprise the majority of mesozooplankton (size range of 0.2-20 mm) biomass and abundance (e.g. Miller *et al.*, 1984; Goldblatt *et al.*, 1999; Mackas and Tsuda, 1999; Coyle and Pinchuk, 2003). As such, they provide an important link between lower trophic levels, including primary producers and microzooplankton, and higher trophic levels such as fish, seabirds, benthic invertebrates, and whales.

This thesis is a portion of a larger study, the U.S. Global Ocean Ecosystem Dynamics (GLOBEC) Northeast Pacific Program (NEP), which endeavors to increase understanding of the physical and biological dynamics of the Gulf of Alaska ecosystem (Weingartner *et al.*, 2002). One of the aims of the NEP GLOBEC program is to determine the secondary production of dominant copepod species within the Gulf of Alaska. Development, growth, and egg production rates are important life history metrics that help determine production of a species. Estimates of secondary production can be used to estimate tertiary production, for example juvenile pink salmon, for a given set of physical and biological conditions. Development and growth of copepods can be determined for *in situ* populations in a variety of ways (Runge and Roff, 2000), but since

growth and development are correlated with temperature and availability of appropriate food (Vidal, 1980a,b; Hirst and Bunker, 2003), it is difficult to determine how much these factors contribute to *in situ* rates. As such, controlling food and temperature conditions in the laboratory allows a predictive capacity as well as a set of conditions to which comparisons to *in situ* rates can be made.

The copepods in this thesis were chosen based on their relative importance in the subarctic Pacific due to seasonally high abundance (Gomez-Gutierrez and Peterson, 1999; Kobari *et al.*, 2003) and relative large size (female prosome lengths of about 1 and 4 mm for *C. abdominalis* and *N. flemingeri*, respectively). However, they have distinct ecological niches. *C. abdominalis* occurs in coastal waters and has highest abundances after the surface water warms, usually in late summer. *N. flemingeri* was recently split from *N. plumchrus* as its own species (Miller, 1988), and so studies previous to 1988 often include a combination of *N. plumchrus* and *N. flemingeri*. *N. flemingeri* is an oceanic species, although it is advected onto the continental shelf (Cooney, 1986), and is adapted to colder waters (Kobari *et al.*, 2003) with development occurring from January to June (Miller and Clemons, 1988). They have distinctly different life cycles. *C. abdominalis* produces multiple generations in a given season (Liang *et al.*, 1996) and surviving unfavorable conditions as diapause eggs (Liang *et al.*, 1994). *N. flemingeri* has an annual life cycle with diapause at depth occurring as females (Miller and Clemons, 1988) in the Gulf of Alaska, although within the western subarctic Pacific, this species has an biennial population with diapause of both copepodite stage four (CIV) and females (Miller and Terazaki, 1989; Tsuda *et al.*, 1999). Egg production, growth, and

development of these species were determined in the laboratory at temperatures typical of the surface layer of the Gulf of Alaska in spring. These metrics were compared to *in situ* populations within their genera as well as other species in the subarctic Pacific. Chapter one provides development and growth of *C. abdominalis* determined from the lower end of its temperature range and looks at trends in development over the thermal range of this species distribution. It also presents *in situ* egg production determined from the Gulf of Alaska. Chapter two provides the first known account of the development of *N. flemingeri* through all development stages as well as growth and fecundity observed at 5°C.

REFERENCES

Cooney, R. T. (1986) The seasonal occurrence of *Neocalanus cristatus*, *Neocalanus plumchrus*, and *Eucalanus bungii* over the shelf of the northern Gulf of Alaska. *Cont. Shelf Res.*, **5**, 541-553.

Coyle, K. O. and Pinchuk, A. I. (2003) Annual cycle of zooplankton abundance, biomass and production on the northern Gulf of Alaska shelf, October 1997 through October 2000. *Fish. Oceanogr.*, **12**, 327-338.

Goldblatt, R. H., Mackas, D. L. and Lewis, A. G. (1999) Mesozooplankton community characteristics in the NE subarctic Pacific. *Deep-Sea Res. II*, **46**, 2619-2644.

Gomez-Gutierrez, J. and Peterson, W.T. (1999) Egg production rates of eight calanoid copepod species during summer 1997 off Newport, Oregon, USA. *J. Plankton Res.*, **21**, 637-657.

Hirst, A. G. and Bunker, A. J. (2003) Growth of marine planktonic copepods: Global rates and patterns in relation to chlorophyll *a*, temperature, and body weight. *Limnol. Oceanogr.*, **48**, 1988-2010.

Kobari, T., Ikeda, T., Kanno, Y., Shiga, N., Takagi, S. and Azumaya, T. (2003) Interannual variations in abundance and body size in *Neocalanus* copepods in the central North Pacific. *J. Plankton Res.*, **25**, 483-494.

Liang, D., Uye, S. and Onbe, T. (1994) Production and loss of eggs in the calanoid copepod *Centropages abdominalis* Sato in Fukuyama Harbor, the Inland Sea of Japan. *Bull. Plankton Soc. Jap.*, **41**, 131-142.

Liang, D., Uye, S. and Onbe, T. (1996) Population dynamics and production of the planktonic copepods in a eutrophic inlet of the Inland Sea of Japan. I. *Centropages abdominalis*. *Mar. Biol.*, **124**, 527-536.

Mackas, D. and Tsuda, A. (1999) Mesozooplankton in the eastern and western subarctic Pacific: community structure, seasonal life histories, and interannual variability. *Prog. Oceanogr.*, **43**, 335-363.

Miller, C.B. (1988) *Neocalanus flemingeri*, a new species of Calanidae (Copepoda: Calanoida) from the subarctic Pacific Ocean, with a comparative redescription of *Neocalanus plumchrus* (Marukawa) 1921. *Prog. Oceanogr.*, **20**, 223-273.

Miller, C. B. and Clemons, M. J. (1988) Revised life history analysis for large grazing copepods in the subarctic Pacific Ocean. *Prog. Oceanogr.*, **20**, 293-313.

Miller, C. B., Frost, B. W., Batchelder, H. P., Clemons, M. J. and Conway, R. E. (1984) Life histories of large, grazing copepods in a subarctic ocean gyre: *Neocalanus plumchrus, Neocalanus cristatus,* and *Eucalanus bungii* in the Northeast Pacific. *Prog. Oceanogr.*, **13**, 201-243.

Miller, C. B. and Terazaki, M. (1989) The life histories of *Neocalanus flemingeri* and *Neocalanus plumchrus* in the Sea of Japan. *Bull. Plankton Soc. Jap.*, **36**, 27-41.

Raymont, J. E. G. (1983) Chapter 2: The major taxa of the marine zooplankton. In *Plankton and productivity in the oceans*. Vol. 2 - Zooplankton. Pergamon Press, New York, pp. 166-229.

Runge, J. A. and Roff, J. C. (2000) The measurement of growth and reproductive rates. In Harris, R. P., Weibe, P. H., Lenz, J., Skjoldal, H. R. & Huntley, M. (eds), *ICES Zooplankton Methodology Manual.* Academic Press, London, pp. 401-454.

Tsuda, A., Saito, H. and Kasai, H. (1999) Life histories of *Neocalanus flemingeri* and *Neocalanus plumchrus* (Calanoida: Copepoda) in the western subarctic Pacific. *Mar. Biol.*, **135**, 533-544.

Vidal, J. (1980a) Physioecology of zooplankton. I. Effects of phytoplankton concentration, temperature, and body size on the growth rate of *Calanus pacificus* and *Pseudocalanus* sp. *Mar. Biol.*, **56**, 111-134.

Vidal, J. (1980b) Physioecology of zooplankton. II. Effects of phytoplankton concentration, temperature, and body size on the development and molting rates of *Calanus pacificus* and *Pseudocalanus* sp. *Mar. Biol.*, **56**, 135-146.

Weingartner, T. J., Coyle, K., Finney, B., Hopcroft, R., Whitledge, T., Brodeur, R., Dagg, M., Farley, E., Haidvogel, D., Haldorson, L., Hermann, A., Hinckley, S., Napp, J., Stabeno, P., Kline, T., Lee, C., Lessard, E., Royer, T. and Strom, S. (2002) The Northeast Pacific GLOBEC Program: Coastal Gulf of Alaska. *Oceanogr.*, **15**, 48-63.

CHAPTER 1

DEVELOPMENT, GROWTH, AND EGG PRODUCTION OF *CENTROPAGES ABDOMINALIS* IN THE EASTERN SUBARCTIC PACIFIC

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ABSTRACT

Centropages abdominalis is a neritic, omnivorous, temporally abundant copepod present throughout the subarctic Pacific and its marginal seas. Development times and growth rates of *C. abdominalis* copepodite stages were determined in the laboratory, and egg production rates were determined for *in situ* populations in the Gulf of Alaska. At 7°C median development time from eggs laid to 50% adults was 42 days and the average weight-specific somatic growth rate was 0.17 d^{-1}, while at 5°C median development time to adult was 59 days (projected) and growth rate averaged 0.08 d^{-1}, suggesting that 5°C is near the lower temperature tolerance limit for this species. The relationship between development time and temperature was established over the range in which this species occurs. The *in situ* growth rate between 10 and 13°C averaged 0.14 d^{-1}, and was generally lower than laboratory growth rates, suggesting that field populations may be food limited during summer and fall.

INTRODUCTION

Copepods in the genus *Centropages* occur in most of the world's oceans and are noted for their raptorial, omnivorous diet. Although phytoplankton and other protists dominate their diet (Conley and Turner, 1985; Turner, 1987; Kleppel, 1993), *Centropages* spp. have been implicated in exerting control on the population dynamics of other copepod species and fish through predation on their eggs (Turner *et al.*, 1985; Sell *et al.*, 2001) and larval stages (Conley and Turner, 1985; Turner, 1987; Davis, 1987).

Thus, improved knowledge of *Centropages* species is valuable, even when they are present in low numbers, because of their predatory impact on other metazoans.

Within the North Pacific, *Centropages abdominalis* is temporally abundant in coastal waters of the subarctic Pacific and its marginal seas. Distribution of this species includes the Inland Sea of Japan (Liang *et al.*, 1994), the Bering and Chukchi Seas (Brodsky, 1950), the Gulf of Alaska (Coyle *et al.*, 1990), and the coastal regions of British Columbia (Mackas, 1992) and Oregon (Gomez-Gutierrez and Peterson, 1999). In the Gulf of Alaska, it is most common in late summer and fall (surface temperature ~12°C), but recruitment can begin during spring at cooler temperatures (~5°C). Despite such widespread occurrence, relatively little is known about the growth and reproduction of this species and the factors that regulate these processes (although see Liang *et al.*, 1994; Liang *et al.*, 1996; Gomez-Gutierrez and Peterson, 1999).

A recurring question in zooplankton research is the extent to which zooplankton growth may be limited by available food. One method used to address this question is comparing development and growth of a species determined in a food and temperature controlled environment to *in situ* populations. The objective of this study was to determine the development and growth rates of *C. abdominalis* in the laboratory at temperatures typical of surface waters in the Gulf of Alaska in spring (5-7°C). By combining observed development times with those from warmer temperatures (Liang *et al.*, 1996), we were able to determine the functional relationship between development time and temperature for the thermal range in which this species is found. *In situ* egg

production rates were determined and subsequently compared to somatic growth rates from the laboratory.

METHOD

Copepods were collected on May 19, 2002 near the Seward Marine Center pier in Resurrection Bay, Alaska (Figure 1, Seward) using a plankton net with 300 µm mesh. Collections were transported to an adjacent lab where ~170 *Centropages abdominalis* females were selected for egg harvesting. Since cannibalism of eggs is reported for this genus (Dagg, 1977; Smith and Lane, 1987), females were placed inside "egg-separation chambers" (Landry, 1983), which consisted of ~500 mL polyvinyl chloride (PVC) tubes with 200 µm Nitex bottoms suspended inside 2 L jars. Female *C. abdominalis* were fed *Isochrysis* sp. and *Pavlova lutheri* (haptophycae, 5-7 µm) and were kept in a Fisher Large Capacity Incubator at 4.9 ± 0.2°C (mean ± S.D.). A total of ~7,800 eggs were harvested over two 24 h intervals and split into nine 8.7 L containers for a final concentration of ~100 eggs L^{-1} as per Fryd *et al.* (Fryd *et al.*, 1991). These containers were divided between two incubators, which maintained temperatures of 4.6 ± 0.3°C and 6.9 ± 0.4°C (mean ± S.D.; hereafter referred to as 5 and 7°C) throughout the rearing as recorded by Onset Tidbit loggers.

Subsamples of ~30 specimens were taken from one or two containers at each temperature every other day throughout the naupliar stages and every day throughout copepodite stages and preserved in 5% formalin. These specimens represent the first ~30 copepods encountered during a systematic scan of material filtered onto a 35 µm mesh

from a volume of water estimated to contain 30 copepods. Population size was estimated at the time of subsample removal and estimates were smoothed using a three-point average. Containers were refreshed by replacing half the water with screened (150 µm) seawater collected from 75 m depth in Resurrection Bay (~33 psu) at ~11 day intervals. The incubators were maintained on a 17:7 h light:dark photoperiod, which approximated the spring photoperiod at this latitude.

Copepods received an addition of a mixture of *Isochrysis* sp. and *Pavlova lutheri* diluted in each container to ~40,000 cells mL^{-1} every third day (± 1 day) beginning after the appearance of the first feeding stage, naupliar stage three (NIII). Feeding was augmented with *in situ* food (10-150 µm) collected in Resurrection Bay to ensure a more nutritionally balanced diet beginning on Day 28; this corresponded to after the appearance of copepodite stage two (CII) at 5°C and CIV at 7°C. Cyanobacteria, ciliates, flagellates, diatoms, eggs, small nauplii, and small larvaceans were observed in varying concentrations in these *in situ* samples. Food concentration was assessed weekly in terms of chlorophyll *a* concentration by filtering a subsample of water from one or two containers at each temperature onto GFF filters. Filters were kept at -40°C until chlorophyll was extracted with 90% acetone, and fluorescence was determined using a Turner Design Model 10 analog fluorometer (Webb *et al.*, 1992). Chlorophyll *a* concentrations were 11 ± 5 mg m^{-3} and 10 ± 2 mg m^{-3} (mean ± S.D.) at 5°C and 7°C, respectively.

Prosome lengths of preserved copepodite stages were measured digitally (n=664 at 5°C and n=550 at 7°C) (ZoopBiom 2.3) (Roff and Hopcroft, 1986). Differences in

length due to temperature, stage, and sex were tested using t-tests. Dry weight was calculated using a published length-weight relationship (Uye, 1982): Log DW = 3.00·Log PL – 7.89, where DW is dry weight (µg), and PL is prosome length (µm). Stage of development was determined using descriptions of *C. typicus* stages as a guide (Lawson and Grice, 1970).

Daily cumulative percent at stage (CPS) was calculated for each preserved subsample. Resolution of naupliar stages was poor, so analyses reflect copepodite stages only. After a stage was observed at 100 CPS, observations of less than 100 CPS were kept only if they occurred for two or more consecutive days. Minimum development time was defined as time to first appearance of a stage. Day 0 was defined as the time that eggs were harvested, when the eggs were ≤ 24 h old. Median development times were determined as days to 50 CPS as predicted by linear regressions of time to CPS (Landry, 1983; Halsband-Lenk *et al.*, 2002). Stage durations were calculated as the difference in median development time between successive stages. Instantaneous growth rates (g) were calculated for each stage from the average dry weight (DW) at a copepodite stage (i) and that stage's duration (D_i): $g_i = (\ln DW_{i+1} - \ln DW_i)\, D_i^{-1}$ (Runge and Roff, 2000).

The relationship between temperature and the median development time of each copepodite stage was predicted using a Bêlehrádek function: $DS = a(T + c)^{-1.58}$, where DS is median development time (day), T is temperature (°C), and a and c are coefficients. The exponent of -1.58 was determined for this species from embryonic development time

(Liang *et al.*, 1994). This relationship incorporates all established development times for *C. abdominalis*, namely this study and one from the Sea of Japan (Liang *et al.*, 1996).

When *C. abdominalis* females were common on the inner continental shelf or within Prince William Sound (Figure 1.1) during Global Ocean Ecosystem Dynamics (GLOBEC) cruises from 2001 - 2003, egg production rates were determined. Zooplankton was collected using a ring net with 200 µm mesh, and 30-48 healthy females were selected for individual incubation in 50 mL Plexiglas towers fitted with 200 µm mesh false-bottoms to prevent egg cannibalism. Egg production experiments were maintained in an incubator at constant near ambient sea-surface temperature under natural photoperiod cycles for 24 h. Individual females and eggs were then preserved and later measured digitally as described previously. Specific egg production rates (SEP) were estimated using female weight predicted from prosome length as described previously and egg weight predicted from measured diameter assuming a density of 0.14 ng C μm^{-3} (Kiorbe *et al.*, 1985) and carbon as 0.40 of dry weight (Båmstedt, 1986).

RESULTS

It took a minimum of 36 days and a median of 42 days for *Centropages abdominalis* to reach adult stages at 7°C (Figure 1.2). Mortality increased dramatically after the appearance of CV at both temperatures (Figure 1.3). Thus, although the first adult was observed after 43 days at 5°C, we were unable to estimate the median development time at this temperature because populations were exhausted prior to reaching 50% adults. However, since it took 51 days to reach median CV at 5°C and

assuming that CIV and CV stage durations are similar (Liang *et al.,* 1996), we project that it would take 59 days to reach median adulthood at 5°C (Figure 1.2). Time to pre-adult stages could be estimated for all stages except CI at 5°C since observations failed to straddle the median for this stage. All remaining linear regressions of time to CPS were significant ($P<0.05$), except for CII at 7°C ($P=0.22$), which was set at midpoint between CI and CIII for subsequent analyses.

Stage durations ranged from 7 to 8 days at 5°C and 3 to 6 days at 7°C (Figure 1.4). Employing our data in conjunction with that of Liang *et al.* (Liang *et al.*, 1996), stage duration decreased with increased temperature (Figure 1.4). The Bêlehrádek equations (Table 1.1) describing the relationship between median development time and temperature produce curves visually consistent with one another (Figure 1.5). These curves were statistically significant ($P<0.05$) for CII and CIII, but marginal ($P<0.10$) for CIV and CV.

Prosome length increased significantly with copepodite stage ($P<0.001$) and males were significantly smaller than females for CV and CVI ($P<0.05$) at both temperatures. CIV, CV female, and CVI male and female at 7°C had significantly greater prosome lengths than these stages at 5°C, but CI was the opposite (Figure 1.6). Average stage-specific instantaneous growth rates were 0.08 d^{-1} and 0.17 d^{-1} at 5°C and 7°C, respectively, and they declined with stage at 7°C (Table 1.2).

C. abdominalis females that produced eggs during the egg production experiments released 56 ± 32 eggs d^{-1} (mean ± S.D.) of diameter 73 ± 4 µm (mean ± S.D.), but in an experiment in early August, 2001, clutch size averaged 100 eggs d^{-1} with

the maximum clutch released by a single female being 180 eggs d^{-1}. Population egg production rates (EPR) averaged 37 ± 22 eggs $female^{-1}$ d^{-1} (mean ± S.D.), with a maximum of 88 eggs $female^{-1}$ d^{-1} and equivalent specific egg production (SEP) rates of 0.14 and 0.37 d^{-1}, respectively (Table 1.3). Thus, prior to correction for temperature differences, somatic and reproductive growth rates span similar ranges.

DISCUSSION

We were successful in establishing the growth rates of *Centropages abdominalis* below the range previously documented and establishing the relationship between development rate and temperature over the thermal range inhabited by this species. The development rate to temperature relationship determined here indicates that median development times for *C. abdominalis* at colder temperatures are less than half of those extrapolated based on development between 10 and 16°C (Liang *et al.*, 1996). Likewise, previous equations that predict size at stage are not applicable at 5°C and overestimate size at 7°C Liang *et al.*, 1996). To some extent, these differences could be a result of an acclimation to colder temperatures, which has been shown to shift Bêlehrádek curves of other copepods (Halsband-Lenk *et al.*, 2002). Alternatively, somatic growth during this study may have been suboptimal for some temperatures and/or stages, resulting in slower rates and less than maximal sizes during our study. Thus, predictions based on warmer temperatures cannot be appropriately projected to the colder temperatures of this species' range.

This study indicates a pronounced difference in prosome length, development time, and growth rate over a narrow temperature range of only 2.3°C. Notably, the larger size of stages at 7°C compared to 5°C is opposite to the trend typical for copepods (Mauchline, 1998), and development times are disproportionately longer at 5°C compared to 7°C. Food type and amount have been shown to have a significant impact on growth of *C. typicus* (Bonnet and Carlotti, 2001). However, because the food regime was similar between our temperatures (if not superior at 5°C due to earlier *in situ* augmentation), we suggest that the stunted development and growth at 5°C is due to this temperature being near the lower tolerance limit for the species. Temperature tolerances and optima are known to exist in other *Centropages* species (Halsband-Lenk *et al.*, 2002). Since we feel that development and growth was sub-optimal at 5°C, subsequent comparisons will be made emphasizing the rates determined at 7°C.

To remove the influence of temperature on growth rates, egg production rates were standardized to 7°C using a Q_{10} of 2.7 for food-satiated adult broadcast spawning copepods (Hirst and Bunker, 2003). The temperature-corrected average *in situ* growth rate of all females, 0.09 d^{-1}, is lower than the average laboratory reared growth rate, 0.17 d^{-1}. This may indicate that adult *C. abdominalis* are food limited in the field. A clear exception to this generalization occurred in August 2001 (Table 1.3), during which the temperature-corrected growth rate of 0.28 d^{-1} was much greater than the average laboratory-reared growth rate. However, if one considers only females that produced eggs during experiments, the average growth of 0.14 d^{-1} was much closer to the laboratory-reared growth rate.

The average laboratory-reared growth rate at 7°C (0.17 d^{-1}) observed in this study is similar to results from previous studies of growth in *C. abdominalis* after correcting temperatures to 7°C as above. The average *in situ* growth rate of females off the coast of Oregon during conditions of upwelling and maximal growth is 0.14 d^{-1} (Gomez-Gutierrez and Peterson, 1999), and in Fukuyama Harbor, Japan is 0.22 d^{-1} (Liang *et al.*, 1994). A previously reported equation relating *in situ* somatic growth rates to temperature for copepodites yields 0.15 d^{-1} at 7°C (Liang *et al.*, 1996). While these rates are comparable to our somatic growth rate, they are higher than the observed population *in situ* growth rate of 0.09 d^{-1}. Egg production rates from our study were similar to rates of *C. abdominalis* from Oregon , where an average of 32 eggs $female^{-1}$ d^{-1} were produced at 10°C (Gomez-Gutierrez and Peterson, 1999). However, egg production was considerably higher in Japan with an average of 109 eggs $female^{-1}$ d^{-1} at 10°C (Liang *et al.*, 1994).

Expanding our view to the genus but ignoring tropical species, there are relatively few other estimates of somatic growth for *Centropages* species, all of which have been determined by laboratory studies. Correcting other studies to 7°C for comparison, we find similar rates to C. *abdominalis* for *C. hamatus* (food-satiated 0.15 d^{-1}) in one study (Klein Breteler *et al.*, 1982), but slightly lower rates in another study (0.11 d^{-1}) (Fryd *et al.*, 1991). Growth rates for *C. typicus* vary from similar (0.14 d^{-1}) (Fryd *et al.*, 1991), to much lower (maximum of 0.07 d^{-1}) (Bonnet and Carlotti, 2001). Overall, *C. abdominalis* appear to have a greater growth rate than *C. typicus* and *C. hamatus*.

There are numerous previous studies describing egg production rates in a wider variety of *Centropages* species. The majority of these somatic and egg production

studies were utilized in a recent synthesis of copepod growth rates (Hirst and Bunker, 2003), which demonstrates growth as a function of temperature and chlorophyll. Based on their Michaelis-Menten relationship (Figure 1.7), maximum growth of *Centropages* spp. at 15°C is 0.60 d^{-1}, with half saturation occurring at 5 mg chlorophyll *a* m^{-3} (Hirst and Bunker, 2003), although there is notable scatter about their relationship (n=192, r^2=0.303, P<0.0001). Converting our data to 15°C using Q_{10} of 2.7, growth rates for most copepodite stages do not follow the relationship of growth to chlorophyll *a* concentrations, with all stages at 5°C and later stages at 7°C expressing lower growth than predicted based on available chlorophyll *a* (Figure 1.7). This disparity suggests that although chlorophyll *a* concentrations should have been adequate for food-saturation, available food size (predominately 5-7 µm) may have been limiting growth. Optimum food size for copepods is thought to be 2-5 % of their prosome length (Berggreen *et al.*, 1988), which means that the main food source during our experiment was below the optimum size beyond naupliar stages, which was also found for *C. typicus* (Bonnet and Carlotti, 2001). While we supplemented with *in situ* food during later copepodite stages, this may still have been inadequate to achieve saturation.

Most of our study's growth rates for female *C. abdominalis* followed the relationship of growth and chlorophyll *a* concentrations, except for some points displaying greater growth (Figure 1.7). These higher growth rates suggest that chlorophyll *a* alone does not always predict food available to these copepods, especially considering their omnivorous diet. However, it is important to note that there was considerable scatter in the data used to generate this curve and that our data may fall

within the natural bounds of this variability. While *Centropages* species have different biogeographic ranges and temperature optima, in general, the growth rates of the various species in this genus appear to respond similarly to temperature and food concentration as measured in terms of chlorophyll *a*.

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REFERENCES

Båmstedt, U. (1986) Chemical composition and energy content. In Corner, E.D.S. and O'Hara, S.C.M. (ed.), *The biological chemistry of marine copepods*. Clarendon, Oxford. pp. 1-58.

Berggreen, U., Hansen, B. and Kiorboe, T. (1988) Food size spectra, ingestion and growth of the copepod *Acartia tonsa* during development: implications for determination of copepod production. *Mar. Biol.*, **99**, 341-352.

Bonnet, D. and Carlotti, F. (2001) Development and egg production in *Centropages typicus* (Copepoda: Calanoida) fed different food types: a laboratory study. *Mar. Ecol. Prog. Ser.*, **224**, 133-148.

Brodskii, K.A. (1950) Calanoida of the far eastern seas and polar basin of the USSR. Academy of Sciences of USSR, Leningrad.

Conley, W. J. and Turner, J. T. (1985) Omnivory by the coastal marine copepods *Centropages hamatus* and *Labidocera aestiva*. *Mar. Ecol. Prog. Ser.*, **21**, 113-120.

Coyle, K. O., Paul, A. J. and Ziemann, D. A. (1990) Copepod populations during the spring bloom in an Alaskan subarctic embayment. *J. Plankton Res.*, **12**, 759-797.

Dagg, M. (1977) Some effects of patchy food environments on copepods. *Limnol. Oceanogr.*, **22**, 99-107.

Davis, C. S. (1987) Components of the zooplankton production cycle in the temperate ocean. *J. Mar. Res.*, **45**, 947-983.

Fryd, M., Haslund, O. H. and Wohlgemuth, O. (1991) Development, growth and egg production of the two copepod species *Centropages hamatus* and *Centropages typicus* in the laboratory. *J. Plankton Res.*, **13**, 683-689.

Gomez-Gutierrez, J. and Peterson, W. T. (1999) Egg production rates of eight calanoid copepod species during summer 1997 off Newport, Oregon, USA. *J. Plankton Res.*, **21**, 637-657.

Kiorboe, T., Mohlenberg, F. and Ulrik riisgard, H. (1985) In situ feeding rates of planktonic copepods: A comparison of four methods. *J. Exp. Mar. Biol. Ecol.*, **88**, 67-81.

Halsband-Lenk, C., Hirche, H.-J. and Carlotti, F. (2002) Temperature impact on reproduction and development of congener copepod populations. *J. Exp. Mar. Biol. Ecol.*, **271**, 121-153.

Hirst, A. G. and Bunker, A. J. (2003) Growth of marine planktonic copepods: Global rates and patterns in relation to chlorophyll *a*, temperature, and body weight. *Limnol. Oceanogr.*, **48**, 1988-2010.

Klein Breteler, W. C. M., Fransz, H. G. and Gonzalez, S. R. (1982) Growth and development of four calanoid copepod species under experimental and natural conditions. *Netherlands J. Sea. Res.*, **6**, 195-207.

Kleppel, G. S. (1993) On the diets of calanoid copepods. *Mar. Ecol. Prog. Ser.*, **99**, 183-195.

Landry, M. R. (1983) The development of marine calanoid copepods with comment on the isochronal rule. *Limnol. Oceanogr.*, **28**, 614-624.

Lawson, T. J. and Grice, G. D. (1970) The developmental stages of *Centropages typicus* Kroyer (Copepoda, Calanoida). *Crustaceana*, **18**, 187-208.

Liang, D., Uye, S. and Onbe, T. (1994) Production and loss of eggs in the calanoid copepod *Centropages abdominalis* Sato in Fukuyama Harbor, the Inland Sea of Japan. *Bull. Plankton Soc. Jap.*, **41**, 131-142.

Liang, D., Uye, S. and Onbe, T. (1996) Population dynamics and production of the planktonic copepods in a eutrophic inlet of the Inland Sea of Japan. I. *Centropages abdominalis*. *Mar. Biol.*, **124**, 527-536.

Mackas, D. L. (1992) Seasonal cycle of zooplankton off Southwestern British Columbia: 1979-89. *Can. J. Fish Aquat. Sci.*, **49**, 903-921.

Mauchline, J. (1998) The biology of calanoid copepods. *Advances in Marine Biology*. Vol. 33. Academic Press, London.

Roff, J. C. and Hopcroft, R. R. (1986) High precision microcomputer based measuring system for ecological research. *Can. J. Fish. Aquat. Sci.*, **43**, 2044-2048.

Runge, J. A. and Roff, J. C. (2000) The measurement of growth and reproductive rates. In Harris, R. P., Weibe, P. H., Lenz, J., Skjoldal, H. R. & Huntley, M. (ed.), *ICES Zooplankton Methodology Manual*. Academic Press, London, pp. 401-454.

Sell, A. F., van Keuren, D. and Madin, L. P. (2001) Predation by omnivorous copepods on early developmental stages of *Calanus finmarchicus* and *Pseudocalanus* spp. *Limnol. Oceanogr.*, **46**, 953-959.

Smith, S. L. and Lane, P. V. Z. (1987) On the life history of *Centropages typicus*: responses to a fall diatom bloom in the New York Bight. *Mar. Biol.*, **95**, 305-313.

Turner, J. T. (1987) Zooplankton feeding ecology: contents of fecal pellets of the copepod *Centropages velificatus* from waters near the mouth of the Mississippi River. *Biol. Bull.*, **173**, 377-386.

Turner, J. T., Tester, P. A. and Hettler, W. F. (1985) Zooplankton feeding ecology: a laboratory study of predation on fish eggs and larvae by the copepods *Anomalocera ornata* and *Centropages typicus*. *Mar. Biol.*, **90**, 1-8.

Uye, S. (1982) Length-weight relationships of important zooplankton from the Inland Sea of Japan. *J. Oceanogr. Soc. Jap.*, **38**, 149-158.

Webb, D.J., Burnison, B.K., Trimbee, A.M. and Prepas, E.E. (1992) Comparison of chlorophyll a extractions with Ethanol and Dimethyl Sulfoxide/Acetone, and a concern about spectrophotometric phaeopigment correction. *Can. J. Fish. Aquat. Sci.*, **49**, 2331-2336.

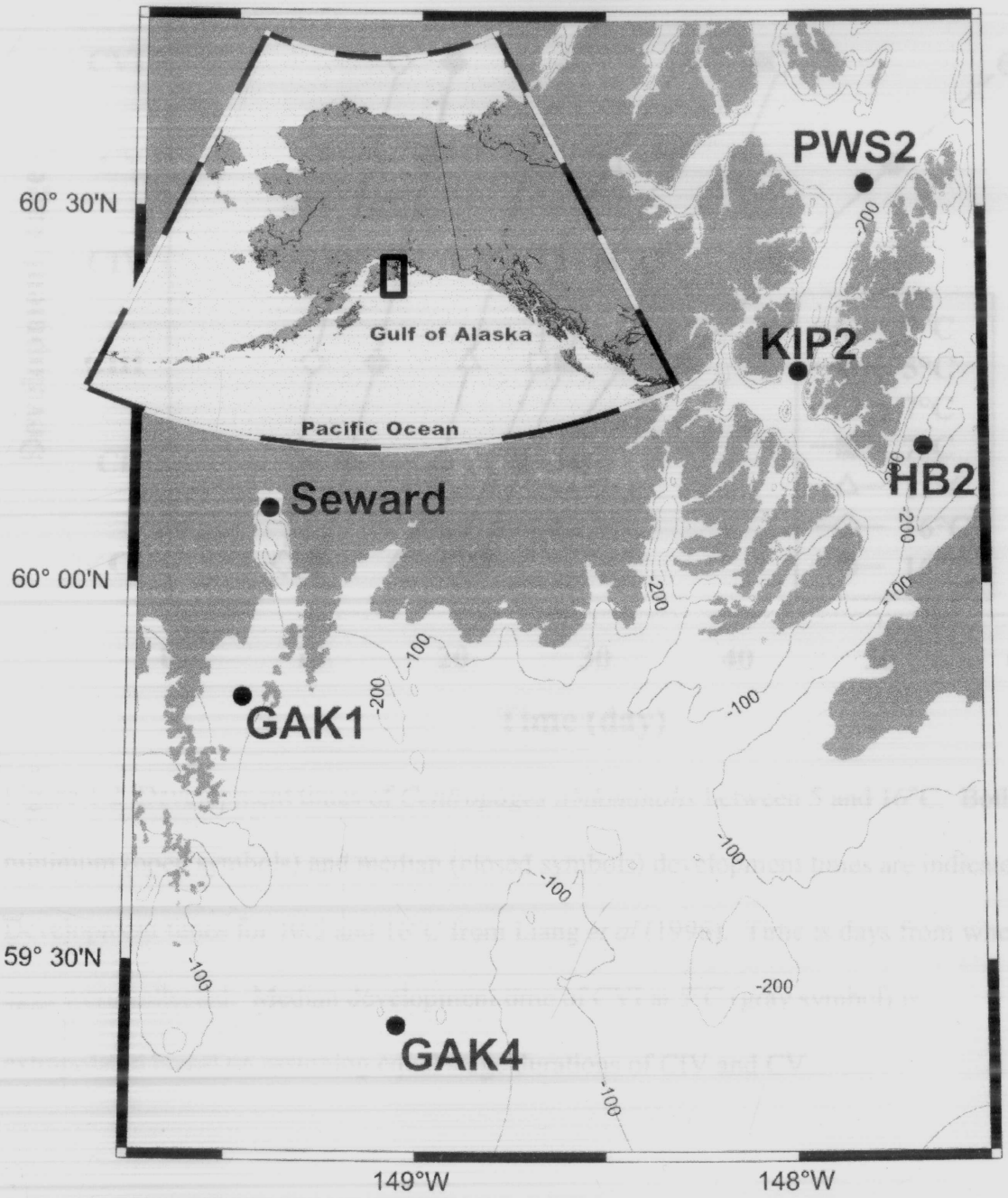

Figure 1.1 Map of sampling locations in the Gulf of Alaska. Figure encompasses the inner continental shelf (GAK1 and GAK4) and Prince William Sound (HB2, KIP2, and PWS2) stations employed for egg production studies. Contour lines show 100 and 200 m depth.

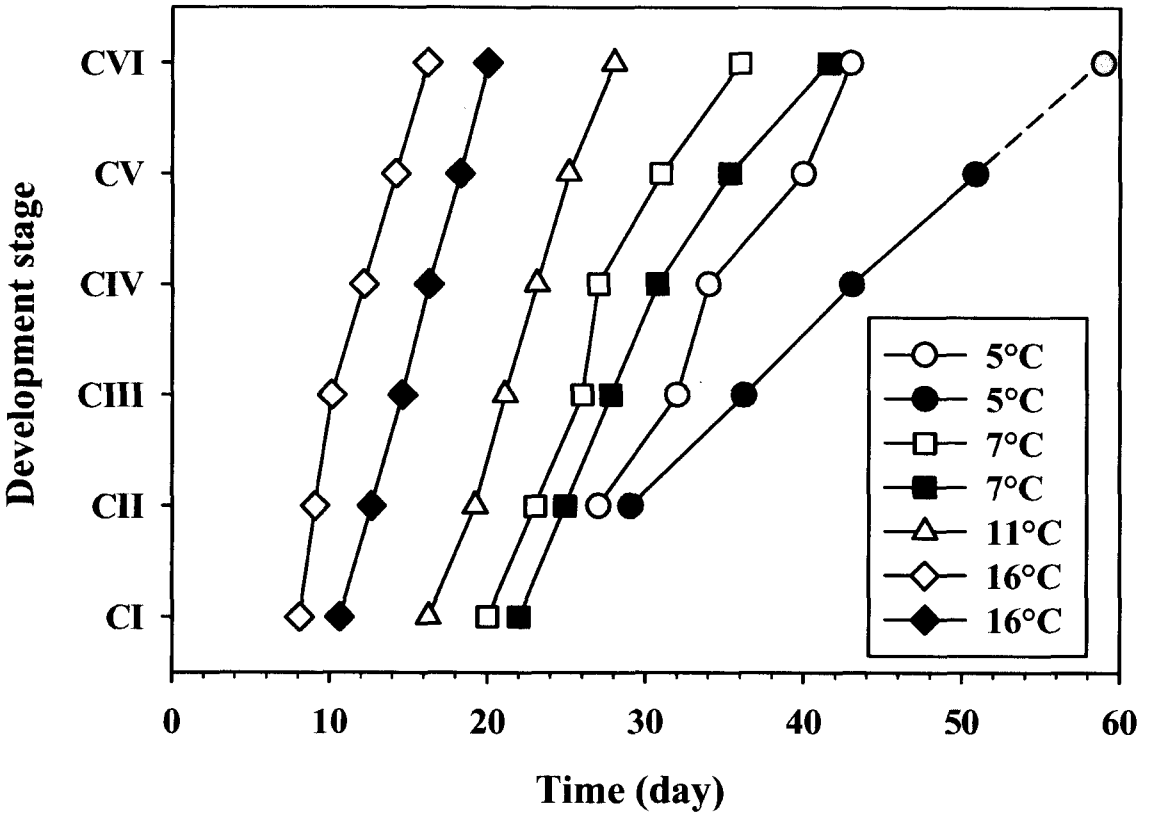

Figure 1.2 Development times of *Centropages abdominalis* between 5 and 16°C. Both minimum (open symbols) and median (closed symbols) development times are indicated. Development times for 10.5 and 16°C from Liang *et al* (1996). Time is days from when eggs were collected. Median development time of CVI at 5°C (gray symbol) is extrapolated based on assuming equal stage durations of CIV and CV.

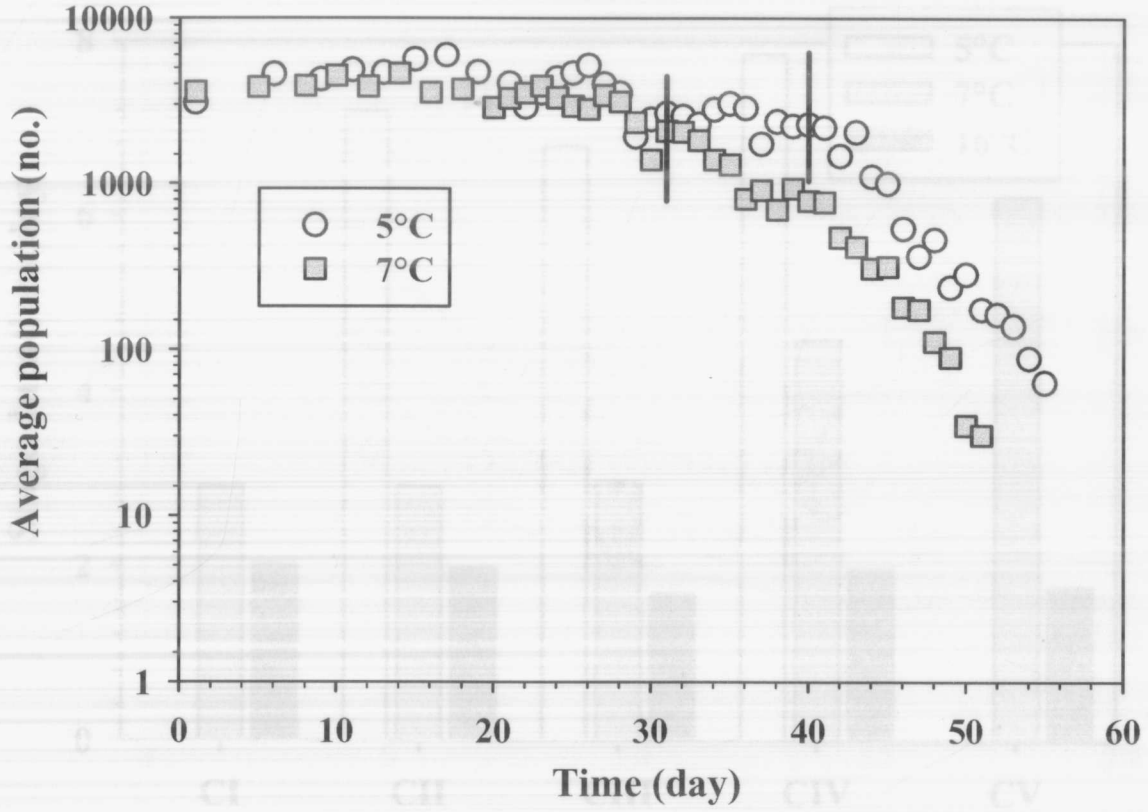

Figure 1.3 Average population of *Centropages abdominalis* used during our study. Vertical lines indicate the first appearance of CV at each temperature. Note that average population is on a log axis and that the greatest decline in populations (i.e. mortality) occurs after the appearance of CV at both temperatures.

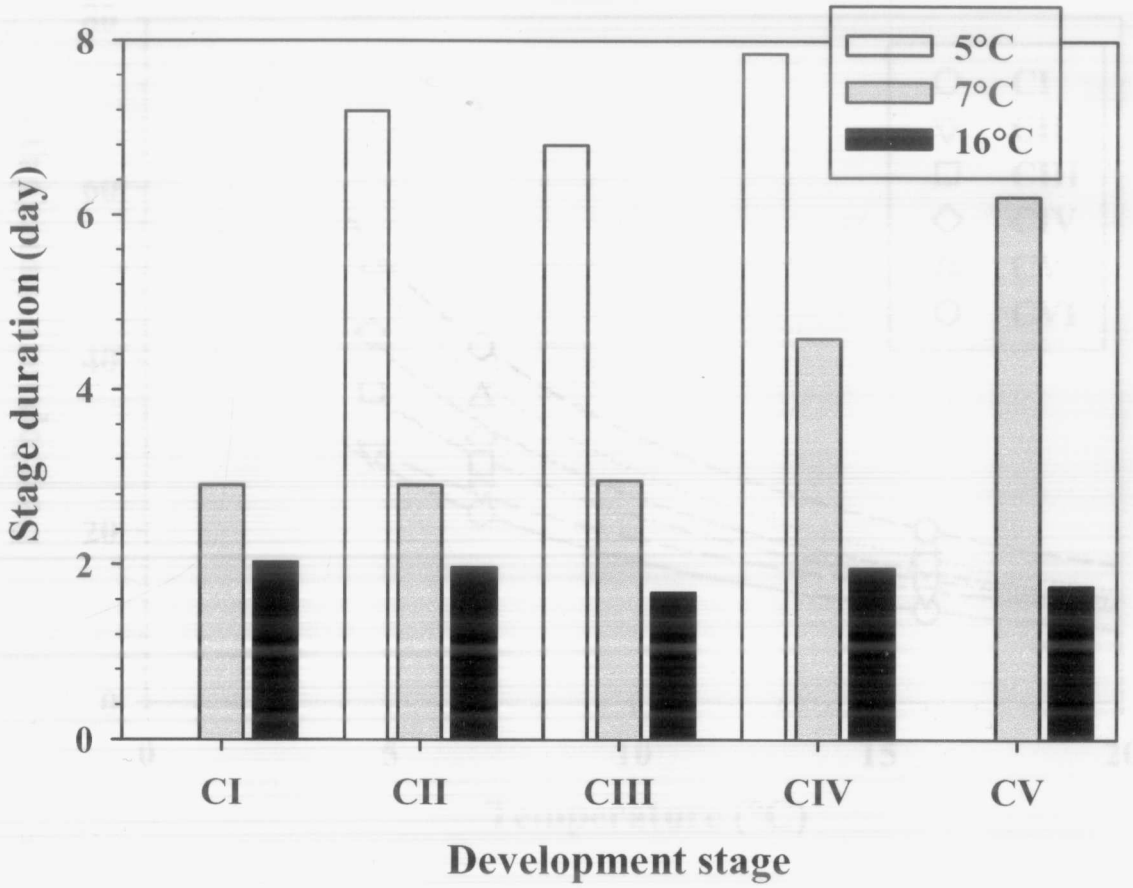

Figure 1.4 Stage durations of *Centropages abdominalis* copepodite stages. Data at 16°C used from Liang *et al.* (Liang *et al.*, 1996).

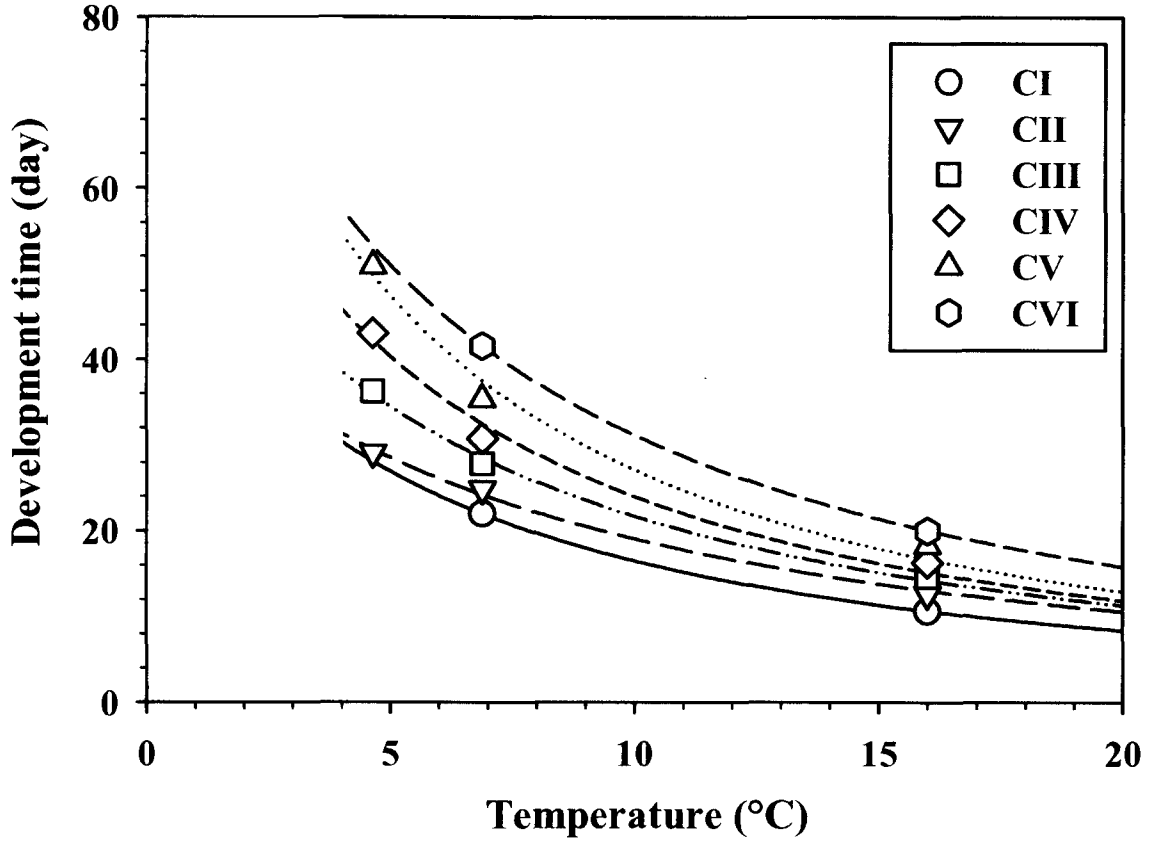

Figure 1.5 Relationship between temperature and median development times of *Centropages abdominalis* copepodite stages. Lines were fit to data using a Bêlehrádek function. Data at 16°C used from Liang *et al.* (1996).

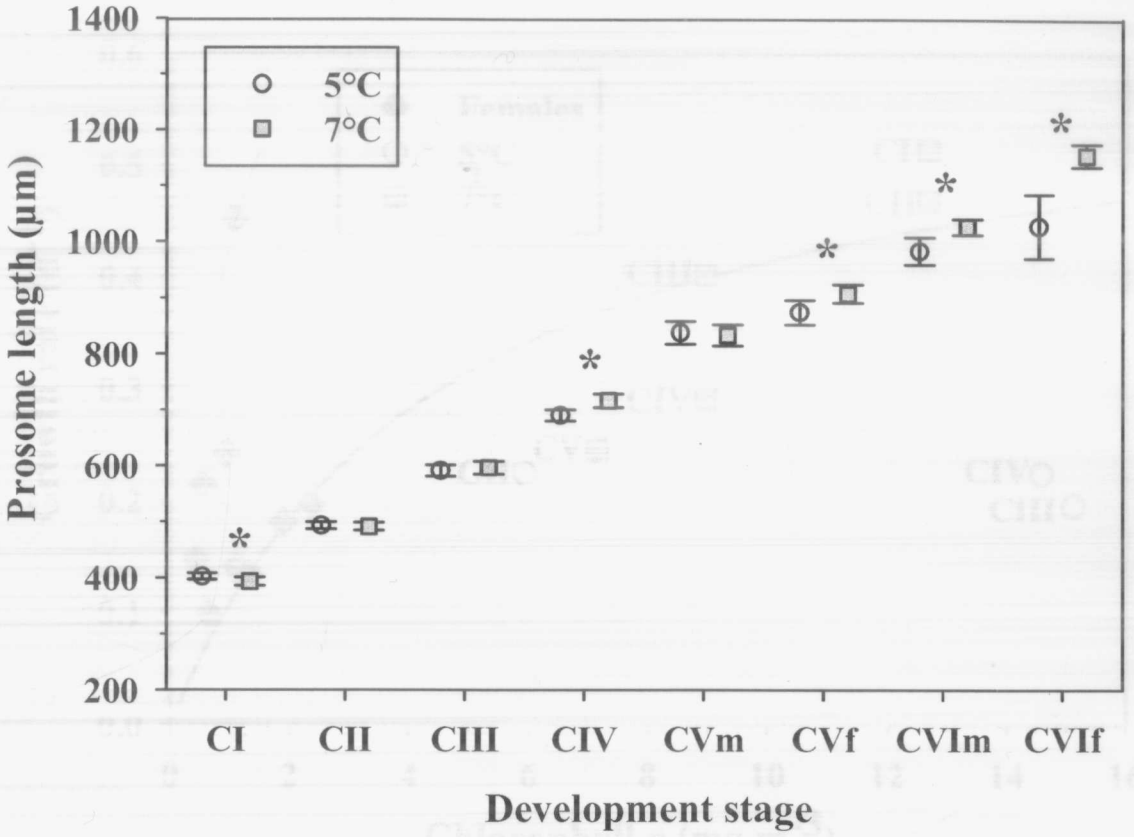

Figure 1.6 Mean prosome lengths of *Centropages abdominalis* reared in the laboratory at 5 and 7°C. Error bars indicate 99% confidence intervals. Within each temperature, length increased significantly with development stage ($P<0.001$) and females were significantly larger than males at CV and CVI ($P<0.05$). Asterisks indicate a significant difference ($P<0.05$) in length of a development stage between temperatures. (n: 16 – 163 at 5°C and 29 – 102 at 7°C.)

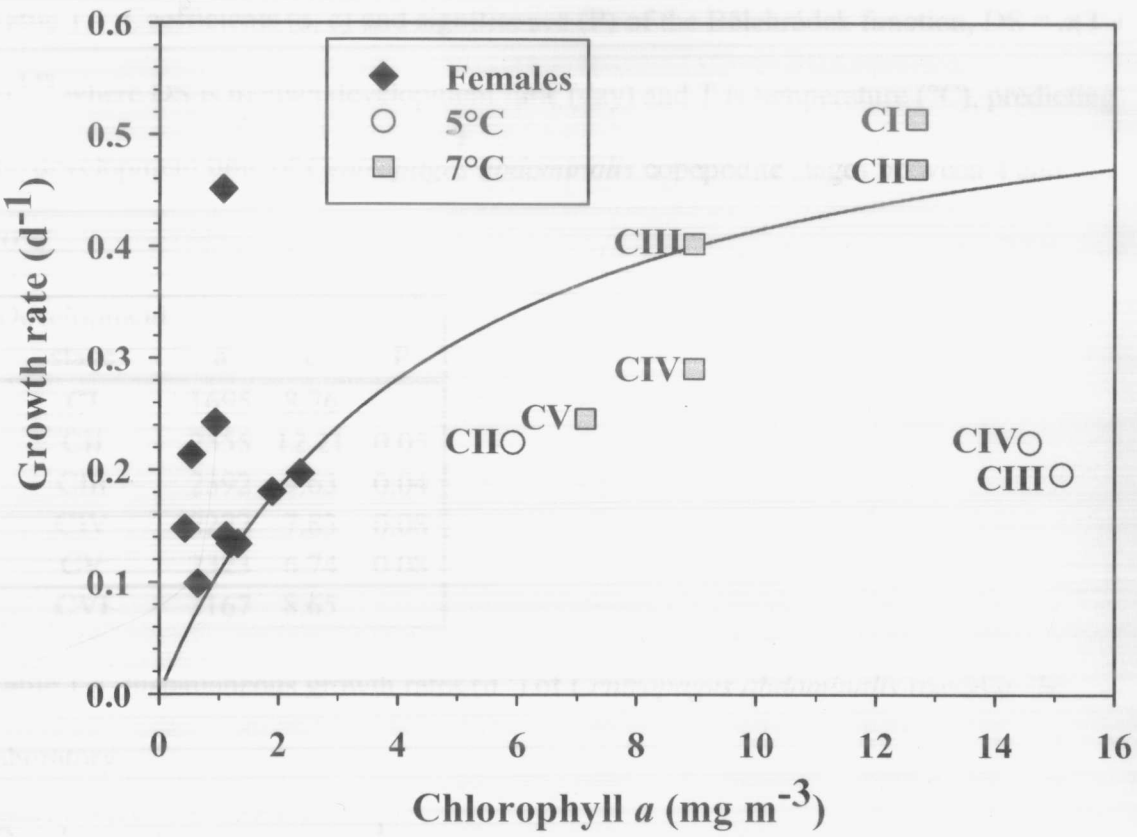

Figure 1.7 Comparison of growth rates to relationship between specific growth rate and chlorophyll *a* concentrations for *Centropages* spp. Growth rates for *in situ* females and laboratory-reared copepodite stages were adjusted to 15°C using a metabolic Q_{10} of 2.7 determined for food-satiated adult broadcast spawning copepods (Hirst and Bunker, 2003). Chlorophyll *a* concentrations are as measured *in situ* (Table 1.3) or within containers near the median development time for each copepodite stage. Line represents Hirst and Bunker's Michaelis-Menton relationship of $g=C_a[g_{max}]\cdot(C_a + K_m)^{-1}$ where g is growth (d^{-1}), C_a is chl *a* concentration (µg Chl *a* L^{-1}), g_{max} is 0.605 ± 0.083 (mean ± S.E.), and K_m is 4.60 ± 1.56 (mean ± S.E.) ($P<0.0001$; $r^2=0.303$; $n=192$) predicted for adult *Centropages* spp. at 15°C (Hirst and Bunker, 2003).

Table 1.1 Coefficients (a, c) and significance (P) of the Bêlehrádek function, DS = $a(T + c)^{-1.58}$ where DS is median development time (day) and T is temperature (°C), predicting the development time of *Centropages abdominalis* copepodite stages between 4 and 20°C.

Development stage	a	c	P
CI	1695	8.76	
CII	2555	12.21	0.05
CIII	2392	9.63	0.04
CIV	2272	7.83	0.08
CV	2323	6.74	0.08
CVI	3167	8.65	

Table 1.2 Instantaneous growth rates (d^{-1}) of *Centropages abdominalis* reared in the laboratory.

Development stage	5°C	7°C
CI		0.23
CII	0.08	0.21
CIII	0.07	0.18
CIV	0.08	0.13
CV		0.11

Table 1.3 Summary of *Centropages abdominalis* egg production from the northern Gulf of Alaska. Sampling date, location (Figure 1), number of females in population (number of egg-layers), mean female prosome length (PL), egg-layer clutch size, population egg production rate (EPR), egg-layer and population specific egg production rates (SEP), and *in situ* temperature and chlorophyll *a* averaged over the upper 40 m of the water column.

Date (y/m/d)	Location	n	PL (μm)	Clutch size	Pop EPR (eggs ♀$^{-1}$ d^{-1})	Egg-layer SEP (d^{-1})	Pop SEP (d^{-1})	T (°C)	Chl. *a* (mg m^{-3})
01/07/04	GAK1	48 (28)	1199	44	28	0.17	0.11	10	1.89
01/07/05	PWS2	40 (27)	1128	31	27	0.13	0.12	10	2.37
01/08/04	GAK1	43 (38)	1133	100	88	0.42	0.37	13	1.07
01/08/07	PWS2	48 (34)	1150	44	31	0.16	0.11	13	1.31
02/10/03	GAK1	24 (21)	1179	59	56	0.19	0.18	12	0.94
03/08/22	HB2	40 (20)	1180	59	29	0.21	0.11	13	1.12
03/10/12	GAK4	61 (24)	1181	67	26	0.22	0.09	10	0.43
03/10/13	GAK1	55 (34)	1156	52	32	0.21	0.13	10	0.54
03/10/16	KIP2	46 (18)	1104	36	14	0.15	0.06	10	0.65

CHAPTER 2

DEVELOPMENT, GROWTH, AND EGG PRODUCTION OF *NEOCALANUS FLEMINGERI* IN THE EASTERN SUBARCTIC PACIFIC

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ABSTRACT

Neocalanus flemingeri is one of the dominant copepods in the subarctic Pacific, yet little is known of its life history traits. Development, growth, and egg production of *N. flemingeri* from the northern Gulf of Alaska were determined in the laboratory at 5°C. Lifetime fecundity of *N. flemingeri* was high with 535 ± 258 eggs female^{-1} (mean ± S.D.). Stage durations varied from 5 to 24 days for NI to CIV, with elongated durations for NIII, NVI, and CIV indicative of the first feeding stage, metamorphosis, and lipid accumulation, respectively. The observed duration of 24 days for NIII is likely overestimated, due to initially inadequate food availability. Median development time from eggs to CV was 117 days. To estimate growth, a length-weight relationship was determined from copepodite stages collected in the field: Log DW = 3.56·Log PL - 2.32, with dry weight (DW) in mg and prosome length (PL) in mm. Weight-specific growth rates of CI to CIV decreased with stage from 0.28 to 0.07 d^{-1} and averaged 0.15 d^{-1}. These results are consistent with observed *in situ* stage durations and growth rates of *N. flemingeri*, as well as *in situ* growth rates of several other copepod species in the northern Gulf of Alaska.

INTRODUCTION

Neocalanus cristatus, *Neocalanus plumchrus*, *Neocalanus flemingeri*, and *Eucalanus bungii* are large grazing copepods in the subarctic Pacific and its marginal seas. They often comprise a majority of the zooplankton biomass, especially in spring and early summer (Miller *et al.*, 1984a; Vidal and Smith, 1986; Tsuda and Sugisaki,

1994; Coyle, 1998; Goldblatt *et al.*, 1999; Coyle and Pinchuk, 2003). *Neocalanus* spp. are important prey for species of juvenile pelagic fish (Willette *et al.*, 1999), myctophid fish (Moku *et al.*, 2000), seabirds (Hunter *et al.*, 2002), and whales (Kawamura 1982).

The subarctic *Neocalanus* species have an annual or biennial life cycle (Mackas and Tsuda, 1999). Their nauplii arrive in the surface layer in early spring, timed to exploit the beginning of the primary production bloom. Growth and development occur in the surface waters until midsummer, followed by vertical migration of CV to depth for diapause, maturation, and mating. Reproduction and egg production occurs during the winter at depth without feeding (Fulton, 1973; Mackas and Tsuda, 1999), and is fueled by the large lipid stores accumulated during the previous summer.

Despite these overall similarities, important differences occur in the life histories of the three species. Until recently, *N. flemingeri* and *N. plumchrus* were grouped as one species (Miller, 1988), and now it is understood that *N. flemingeri* occurs in the upper water column earlier than *N. plumchrus* (Miller and Clemons, 1988; Tsuda *et al.*, 1999; Kobari and Ikeda, 2001a, b). The vertical distribution of *Neocalanus* spp. within the surface layer of the subarctic Pacific varies, with *N. cristatus* occurring lower in the water column than *N. flemingeri* and *N. plumchrus* (Mackas *et al.*, 1993), although this separation is not apparent in the Bering Sea (Coyle, 1998). Of the three species, *N. flemingeri* is often observed to be more common within inner shelf waters than the other two species (Coyle and Pinchuk, in press).

Despite numerous studies involving subarctic *Neocalanus* spp. (e.g. Miller *et al.*, 1984a; Miller and Clemons, 1988; Miller and Nielsen, 1988; Miller and Terazaki, 1989;

Miller *et al.*, 1992; Miller, 1993) there is relatively little known of the development and growth of any species, but especially for *N. flemingeri.* Of the three subarctic *Neocalanus* species, growth or development rates of the copepodite stages have only been directly determined for *in situ* populations of *N. plumchrus* (Miller and Nielsen, 1998; Miller 1993) or a mixed assemblage of *N. plumchrus* and *N. flemingeri* (Vidal and Smith, 1986). Egg production and early development of the three *Neocalanus* species have received only limited study, and the development past naupliar stage three (NIII) has not been observed for *N. flemingeri* and *N. plumchrus* (Saito and Tsuda, 2000). Here we report on the egg production, development time and growth rates of *N. flemingeri* to late copepodite stages based on laboratory rearing.

METHOD

Rearing of copepods was divided into two methodologies: the first consisted of determining the development from eggs to stage CII by means of daily population subsampling and estimates of cumulative percentage at stage (CPS) and the second determined stage durations of CII to CIV by checking daily for molts of individual copepodites. Temperature was maintained at 4.9 ± 0.8°C (mean ± S.D.) throughout the laboratory rearing.

EGG PRODUCTION AND EARLY DEVELOPMENT

Adult *Neocalanus flemingeri* were collected in Prince William Sound, Alaska (60°32"N 147°48"W) at 400-600 m water depth in mid-December, 2002 using a 100 µm MOCNESS net. Approximately 150 healthy females (with gonads that did not show

advanced oogenesis) were removed from the collections and divided between three 20 L carboys filled with water from the collection depth. They were transported to a laboratory in Fairbanks and then 35 females were placed individually into 1 L polyethylene terephthalate copolyester (PETG) bottles. The females held singly were checked every 3-4 days for eggs beginning in January, and any eggs (or nauplii) present were removed, counted, and preserved. After females were spent (Miller and Clemons, 1988), they were preserved. Females that failed to produce eggs (n=3) or died prior to spawning-out (n=3) were removed from analysis.

Once single females started to release eggs in early January, 2003, females from the remaining carboys were placed inside "egg-separation chambers" (Landry, 1983), which consisted of ~500 mL polyvinyl chloride (PVC) tubes with 200 µm Nitex bottoms suspended inside 2 L jars. Eggs were harvested every 24 h for 3 days, during which ~8300 eggs were produced and distributed into multiple 8.7 L containers at concentrations of ~68 eggs L^{-1}. Each day a volume of water containing approximately 30 copepods was removed from a container after gentle mixing and subsamples of ~30 individuals were preserved in a 5% buffered formalin solution until populations were nearly exhausted (~3% of initial population). Day 0 was defined as the time that eggs were harvested, when the eggs were ≤ 24 h old.

Copepods were kept in the dark until after the appearance of NIII, at which point a light:dark cycle was initiated and periodically adjusted to mimic local conditions in the northern Gulf of Alaska. Cultures received an addition of a mixture of *Isochrysis* sp. and *Pavlova lutheri* (haptophycae, 5-7 µm) diluted in each container to ~40,000 cells mL^{-1} on

Day 34 and again 13 days later. Cumulative mortality was high, so after Day 50 subsampled nauplii (~30 d^{-1}) were placed individually in 10 mL multi-well tray wells. On Day 55 all remaining copepods were removed and placed in wells (total n=280). Stage of development was determined daily for all *N. flemingeri* in wells, and individuals were preserved upon natural death. Copepods received a mixture of *Isochrysis* sp. and *Pavlova lutheri* diluted in each well to ~260,000 cells mL^{-1} once placed in multi-well trays, and every four days thereafter.

Preserved copepods were measured digitally (ZoopBiom 2.2) (Roff and Hopcroft, 1986), and stage of development was determined using descriptions of *Neocalanus gracilis* as a guide (Sazhina, 1985). Lengths used for NV and NVI were determined from preserved individuals during the non-destructive subsampling. Differences in lengths between all stages were examined using t-tests. Cumulative percentage at stage (CPS) was determined for each elapsed day; stages that comprised more than 95 CPS for two consecutive days were set at 100 CPS on the following day. Eggs were assumed unviable three days after the majority hatched and were not included in further calculations. Least squares linear regressions of CPS were used to calculate median development times (Landry, 1983) and stage durations of NI to CI were calculated as the difference between median development times of successive stages. Estimations of population size were made during daily subsampling and were smoothed using a three-point average; estimates from containers in which populations appeared unhealthy were discarded.

STAGE DURATION AND GROWTH OF CII TO CIV

Zooplankton was collected in Prince William Sound, Alaska (60°17'N 147°59'W) from 50 m to the surface on March 3, 2003 using a 64 μm ring net. *Neocalanus flemingeri* CI were selected, placed in filtered seawater with additions of cultured *Isochrysis* sp., *Pavlova lutheri*, and *Chromonas* sp., and transported to the Fairbanks laboratory. *N. flemingeri* (n=366) were placed in individual 70 mL flasks and checked daily for stage; after each copepod molted to CIII, it was transferred to a 270 mL flask. Since development to adulthood seems to occur during *N. flemingeri*'s descent from the surface layer (Miller and Clemons, 1988) and we could not mimic such conditions in the laboratory, observations of copepods stopped after molt to CV.

Copepods received an addition of a mixture of *Isochrysis* sp. and *Pavlova lutheri* diluted in each container to ~40,000 cells mL^{-1} every 6 days throughout the experimental period. In order to increase the nutritional value of the food source, a mixture of *Thalassiosira*, *Rhodomonas*, *Ditylum*, *Cosconodiscus*, *Gymnodinium*, and *Prorocentrum* were added when they became available and accounted for up to 12% of the food mixture during the final 18 days of the rearing. Total water changes using filtered (35 μm) seawater from the northern Gulf of Alaska (S ~33psu) occurred every ~10 days. For those few days (n=5) that checks did not occur, stage changes on the following day were attributed equally between that day and the previous day.

Stage duration was calculated as the median number of days between stages (n=176, 104, and 50 for stages CII, CIII, and CIV respectively). Instantaneous growth rates (g) were calculated from the predicted dry weight (DW) of the mean length for each

copepodite stage (i) and that stage's duration (D_i): $g_i = (\ln DW_{i+1} - \ln DW_i) D_i^{-1}$ (Runge and Roff, 2000). Copepodite dry weights were predicted from a length-weight relationship created with *N. flemingeri* from the northern Gulf of Alaska: Log DW = 3.56·Log PL - 2.32 (R^2=0.95, n=114), where DW is dry weight (mg) and PL is prosome length (mm) (Figure 2.1). This length-weight relationship was determined by digitally measuring copepod prosome lengths, placing the copepods in pre-weighed pans set in a drying oven at 55°C for 24 h, and then weighing dry weight to ± 0.1 µg using a Cahn Microbalance. Single, live copepods were used for each length-weight measurement for CIII to CV. Smaller stages were immobilized with several drops of formalin immediately before measurement and groups of ten CI and pairs of CII with lengths within 25 µm ranges were used. Prosome lengths of CI to CV were obtained from samples collected in the northern Gulf of Alaska during March, April and May of 2002.

RESULTS

Lifetime fecundity of *Neocalanus flemingeri* was high with 535 ± 258 eggs female^{-1} (mean ± S.D.) and a maximum of 1036 eggs female^{-1}. Spent females contained little remaining somatic tissue. Unfortunately, some initial clutches are likely combined because all females laid eggs prior to the first observation in January. In instances (n=9) during this first observation involving a mixture of both nauplii and undeveloped eggs, these entities were divided into 2 clutches. Without further accounting for this source of error, up to 4 clutches of eggs could be identified with an average clutch size of 225 ±

154 eggs (mean ± S.D.), and a trend of decreasing clutch size with successive clutches (Figure 2.2). All females were spent by the end of January.

Egg hatching was nearly synchronous with >90% of the eggs hatching between Day 2 and 3. Development of stages was also relatively synchronous and least-squares regressions of CPS were significant ($P<0.005$) except for NI ($P=0.16$) and CII ($P=0.06$) (Figure 2.3). There was a marked increase in development after the change of methodology, perhaps due to increased handling (Miller *et al.*, 1984b). Median development time of *N. flemingeri* to CI was 73 days (Figure 2.3). Based on development time to CI in conjunction with stage durations of CI to CIV (Table 2.1), we estimate median development time from eggs lain to CV as 117 days.

Length increased with development stage ($P<0.001$), except for NII and NIII ($P=0.87$) (Figure 2.4). Stage durations were between five and eight days for NI, NIV, NV, and CI and longer for other stages (Table 2.1). NIII had the longest stage duration, followed by NVI and CIV. Weight-specific growth rates of CI to CIV averaged 0.15 d^{-1} and decreased with stage from 0.28 to 0.07 d^{-1} (Table 2.1).

Mortality of copepods during incubation was low until the population reached 50 CPS NIII, after which there was a population loss of ~92% by 50 CPS NIV. Mortality was still high during NIV with a loss of ~67% of the remaining population. After methodology was changed to non-destructive subsampling, mortality was still high and averaged 5% d^{-1}.

DISCUSSION

A previous study of egg production reported a greater fecundity for *N. flemingeri* and *N. plumchrus* with 924 ± 346 (mean ± S.E.) eggs female^{-1} and 840 ± 214 (mean ± S.E.) eggs female^{-1}, respectively (Saito and Tsuda, 2000). Although the prosome length of female *N. flemingeri* reported in that study was identical to our study, their estimate was restricted to females laying at least 4 clutches and likely represents an overestimation for the general population. Alternatively, the females in their study may have accumulated greater lipid reserves than those in our study. The only other study of egg production for these species reports fecundity of [*Neo*]*Calanus plumchrus* averaging 535 eggs female^{-1} (Fulton, 1973), which is similar to our results. However, in both Fulton's study and ours, females were deliberately selected with immature ovaries, so it is possible that both these estimates are not reflective of the total population (Fulton, 1973).

Due to problems with phytoplankton cultures, copepods were not fed until 8 days after populations reached 100 CPS NIII, and the first NIV was not seen until 4 days later. The absence of NIV prior to the introduction of food supports previous observations that NIII must be the first feeding stage and that development to NIV cannot occur in the absence of food (Saito, Shiogama, personal communication). Although the first feeding naupliar stage is typically protracted compared to adjoining stages (Mauchline, 1998), because the first feeding was inadvertently delayed until 21 days after the first appearance of NIII, our NIII duration is likely longer and mortality within the stage greater than if feeding had occurred throughout NIII.

Overall, development times were similar to previous studies of *N. flemingeri* and *N. plumchrus*. Our egg hatching time was slightly faster than that reported at 4°C (Saito and Tsuda, 2000). Larval development time was similar to the estimated development time for *N. plumchrus* of 55 days [5 day egg duration and 10 days for each naupliar stage (Mackas and Tsuda, 1999)]. Development to CV was similar to the development time of ~120 days from hatching to CV determined in a similar study of *N. flemingeri* reared at 5°C, although that study also observed a long NIII stage duration and acknowledge that food conditions may have been inadequate during that stage (Ban and Takayama, Shiga, personal communication). An estimation of the development times of *N. flemingeri* deduced from the mean relative development time of *Calanus* spp. suggested 38 days for hatching to CI and 49 days from CI to CV (Saito and Tsuda, 2000). The first estimation is nearly half of our median development time to CI (73 days) (Figure 2.3), while the second estimation is only five days longer than our median development time from CI to CV (44 days). This discrepancy could be due to an inherently longer juvenile development of *N. flemingeri* compared to *Calanus* spp., but is probably impacted by our large estimate of NIII stage duration.

Long stage durations for NIII, NVI, and CIV are consistent with prolonged development due to increased energy demands associated with a first feeding stage, metamorphosis, and the accumulation of lipid stores, respectively (Mauchline, 1998). Copepodite stage durations are in good agreement with those of *N. plumchrus* (including *N. flemingeri*) estimated from *in situ* populations in the Bering Sea [8, 10, 12, 16 days for CI-CIV, respectively (Vidal and Smith, 1986)]. Initial *in situ* estimations of stage

durations of *N. plumchrus* in the Alaskan gyre were nearly double our estimates at 24 and 25 days for CIII and CIV (Miller and Nielsen, 1988). However, a later estimate corrected for unequal mortality with use of a model, and predicted stage durations of 13 days each for CI to CIII and 17 days for CIV (Miller, 1993). These later estimates compare well with our results, except for CI, for which our duration is notably shorter.

Since the lengths of NV and NVI were taken from individuals after death, they may have been smaller than representative of the population. Mean prosome lengths for CII to female in this study were within the standard deviation of the mean prosome length reported from the Oyashio region (Kobari *et al.*, 2003). Both the y-intercept and slope of our length-weight relationship are slightly lower but in general comparable to those of a length-weight relationship reported from the Oyashio region (Kobari *et al.*, 2003).

N. flemingeri copepodite growth rates are substantial and suggest a body weight doubling time of less than three days for CI and nearly 10 days for CIV. *In situ* growth rates for these same stages of *N. plumchrus* (including *N. flemingeri*) in the Bering Sea also decreased with stage, although over a much narrower range of 0.14 – 0.11 d^{-1} (Vidal and Smith, 1986), of which there was close agreement with our rates for CII and CIII. Even though reported stage durations for *N. plumchrus* CIII and CIV were longer than ours, their growth rate of 0.05 d^{-1} for CIV is similar to ours (Miller and Nielsen, 1988). Our laboratory-determined stage durations and growth rates are in good agreement with *in situ* values for *N. flemingeri* in the northern Gulf of Alaska during April and May in which stage durations of CI to CIV were ~10 d and growth rate decreased with stage from ~0.13 to ~0.07 d^{-1} (Liu, Clarke, and Hopcroft, Fairbanks, personal communication).

Several reasons could be postulated for the prominence of *Neocalanus* species biomass in the subarctic. One logical hypothesis would be that they simply grow faster than co-occurring species and therefore are more successful. Growth rates of *N. flemingeri*, however, appear consistent with other copepod species in the Gulf of Alaska. For example, *Centropages abdominalis* has an average copepodite growth rate of 0.17 d^{-1} (Slater and Hopcroft, in review), *Pseudocalanus newmani* and *P. mimus* have *in situ* specific egg production rates of ~0.12 and ~0.09 d^{-1}, respectively (Hopcroft and Clarke, Fairbanks, personal communication), and *Metridia pacifica* and *M. okhotensis* have *in situ* specific egg production rates of 0.10 and 0.11 d^{-1}, respectively (Hopcroft *et al.*, in press). Although it has been suggested that *N. flemingeri* has the largest clutch size of any calanoid copepod (Saito and Tsuda, 2000), there is no data indicating that their lifetime fecundity is substantially different from other species. It is most likely that their role as prominent contributors to spring biomass in the subarctic Pacific has more to do with their large size and other life history traits than with their growth rates.

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REFERENCES

Coyle, K. (1998) *Neocalanus* scattering layers near the western Aleutian Islands. *J. Plankton Res.*, **20**, 1189-1202.

Coyle, K. O. and Pinchuk, A. I. (2003) Annual cycle of zooplankton abundance, biomass and production on the northern Gulf of Alaska shelf, October 1997 through October 2000. *Fish. Oceanogr.*, **12**, 327-338.

Coyle, K. O. and Pinchuk, A. I. (in press) Cross-shelf distribution of zooplankton relative to water masses on the northern Gulf of Alaska shelf. *Deep-Sea Res. II.*

Fulton, J. (1973) Some aspects of the life history of *Calanus plumchrus* in the Strait of Georgia. *J. Fish. Res. Bd. Can.*, **30**, 811-815.

Goldblatt, R. H., Mackas, D. L. and Lewis, A. G. (1999) Mesozooplankton community characteristics in the NE subarctic Pacific. *Deep-Sea Res. II*, **46**, 2619-2644.

Hopcroft, R. R., Clarke, C., Byrd, A. G. and Pinchuk, A. I. (in press) The paradox of *Metridia* spp. egg production rates: A new technique and measurements from the coastal Gulf of Alaska. *Mar. Ecol. Prog. Ser.*

Hunter, F. M., Jones, I. L., Williams, J. C. and Byrd, G. V. (2002) Breeding biology of the Whiskered Auklet (*Aethia pygmaea*) at Buldir Island, Alaska. *The Auk*, **119**, 1036-1051.

Kawamura, A. (1982) Food habits and prey distributions of three rorqual species in the North Pacific Ocean. *Sci. Rep. Whales Res. Inst.*, **34**, 59-91.

Kobari, T. and Ikeda, T. (2001a) Life cycle of *Neocalanus flemingeri* (Crustacea:Copepoda) in the Oyashio region, western subarctic Pacific, with notes on its regional variations. *Mar. Ecol. Prog. Ser.*, **209**, 243-255.

Kobari, T. and Ikeda, T. (2001b) Ontogenetic vertical migration and life cycle of *Neocalanus plumchrus* (Crustacea: Copepoda) in the Oyashio region, with notes on regional variations in body sizes. *J. Plankton Res.*, **23**, 287-302.

Kobari, T., Shinada, A. and Tsuda, A. (2003) Functional roles of interzonal migrating mesozooplankton in the western subarctic Pacific. *Prog. Oceanogr.*, **57**, 279-298.

Landry, M. R. (1983) The development of marine calanoid copepods with comment on the isochronal rule. *Limnol. Oceanogr.*, **28**, 614-624.

Mackas, D. and Tsuda, A. (1999) Mesozooplankton in the eastern and western subarctic Pacific: community structure, seasonal life histories, and interannual variability. *Prog. Oceanogr.*, **43**, 335-363.

Mackas, D. L., Sefton, H., Miller, C. B. and Raich, A. (1993) Vertical habitat partitioning by large calanoid copepods in the oceanic Subarctic Pacific during spring. *Prog. Oceanogr.*, **32**, 259-294.

Mauchline, J. (1998) The biology of calanoid copepods. *Advances in Marine Biology*. Vol. 33. Academic Press, London.

Miller, C. B. (1988) *Neocalanus flemingeri*, a new species of Calanidae (Copepoda: Calanoida) from the subarctic Pacific Ocean, with a comparative redescription of *Neocalanus plumchrus* (Marukawa) 1921. *Prog. Oceanogr.*, **20**, 223-273.

Miller, C. B. (1993) Development of large copepods during spring in the Gulf of Alaska. *Prog. Oceanogr.*, **32**, 295-317.

Miller, C. B. and Clemons, M. J. (1988) Revised life history analysis for large grazing copepods in the subarctic Pacific Ocean. *Prog. Oceanogr.*, **20**, 293-313.

Miller, C. B., Frost, B. W., Batchelder, H. P., Clemons, M. J. and Conway, R. E. (1984a) Life histories of large, grazing copepods in a subarctic ocean gyre: *Neocalanus plumchrus, Neocalanus cristatus,*and *Eucalanus bungii* in the Northeast Pacific. *Prog. Oceanogr.*, **13**, 201-243.

Miller, C. B., Fulton, J. and Frost, B. W. (1992) Size variation of *Neocalanus plumchrus* and *Neocalanus flemingeri* in a 20-yr sample series from the Gulf of Alaska. *Can. J. Fish. Aquat. Sci.*, **49**, 389-399.

Miller, C. B., Huntley, M. E. and Brooks, E. R. (1984b) Post-collection molting rates of planktonic, marine copepods: Measurement, applications, problems. *Limnol. Oceanogr.*, **29**, 1274-1289.

Miller, C. B. and Nielsen, R. D. (1988) Development and growth of large, calanid copepods in the ocean subarctic Pacific, May 1984. *Prog. Oceanogr.*, **20**, 275-292.

Miller, C. B. and Terazaki, M. (1989) The life histories of *Neocalanus flemingeri* and *Neocalanus plumchrus* in the Sea of Japan. *Bull. Plankton Soc. Jap.*, **36**, 27-41.

Moku, M., Kawaguchi, K., Watanabe, H. and Ohno, A. (2000) Feeding habits of three dominant myctophid fishes, *Diaphus theta*, *Stenobrachius leucopsarus* and *S. nannochir*, in the subarctic and transitional waters of the western North Pacific. *Mar. Ecol. Prog. Ser.*, **207**, 129-140.

Roff, J. C. and Hopcroft, R. R. (1986) High precision microcomputer based measuring system for ecological research. *Can. J. Fish. Aquat. Sci.*, **43**, 2044-2048.

Runge, J. A. and Roff, J. C. (2000) The measurement of growth and reproductive rates. In Harris, R. P., Weibe, P. H., Lenz, J., Skjoldal, H. R. & Huntley, M. (eds), *ICES Zooplankton Methodology Manual.* Academic Press, London, pp. 401-454.

Saito, H. and Tsuda, A. (2000) Egg production and early development of the subarctic copepods *Neocalanus cristatus*, *N. plumchrus* and *N. flemingeri. Deep-Sea Res. I.*, **47**, 2141-2158.

Sazhina, L. I. (1985) *Nauplii of the major species of pelagic copepods of the world oceans*. Naukova Dumka Press, Kiev. (In Russian)

Slater, L. M. and Hopcroft, R. R. (in review) Development, growth, and egg production of *Centropages abdominalis* in the eastern subarctic Pacific.

Tsuda, A. and Sugisaki, H. (1994) *In situ* grazing rate of the copepod population in the western subarctic North Pacific during spring. *Mar. Biol.*, **120**, 203-210.

Tsuda, A., Saito, H. and Kasai, H. (1999) Life histories of *Neocalanus flemingeri* and *Neocalanus plumchrus* (Calanoida: Copepoda) in the western subarctic Pacific. *Mar. Biol.*, **135**, 533-544.

Vidal, J. and Smith, S. L. (1986) Biomass, growth and development of populations of herbivorous zooplankton in the southeastern Bering Sea during spring. *Deep-Sea Res.*, **33**, 523-556.

Willette, T. M., Cooney, R. T. and Hyer, K. (1999) Predator foraging mode shifts affecting mortality of juvenile fishes during the subarctic spring bloom. *Can. J. Fish. Aquat. Sci.*, **56**, 364-376.

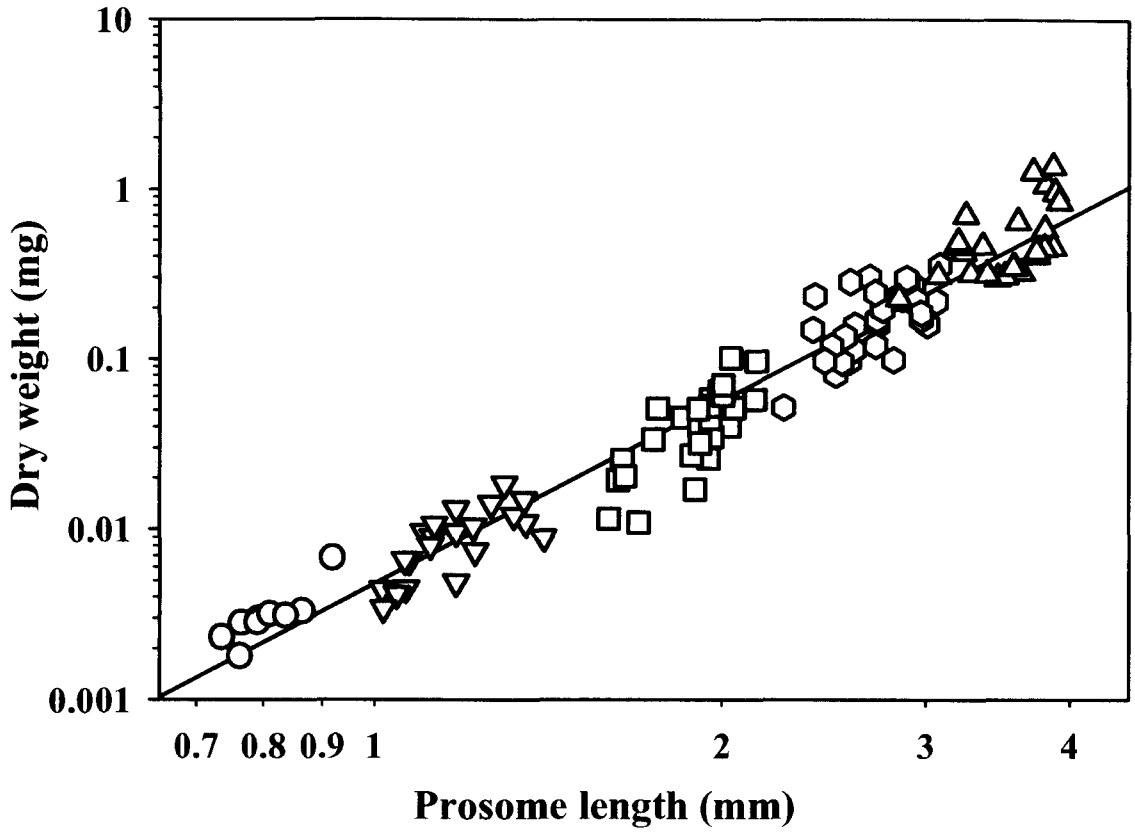

Figure 2.1 Relationship between prosome length (PL, mm) and dry weight (DW, mg) for *Neocalanus flemingeri* CI to CV (depicted by different symbols). Fitted regression line gives relationship of: Log DW = 3.56·Log PL - 2.32 (n=114, R^2=0.95).

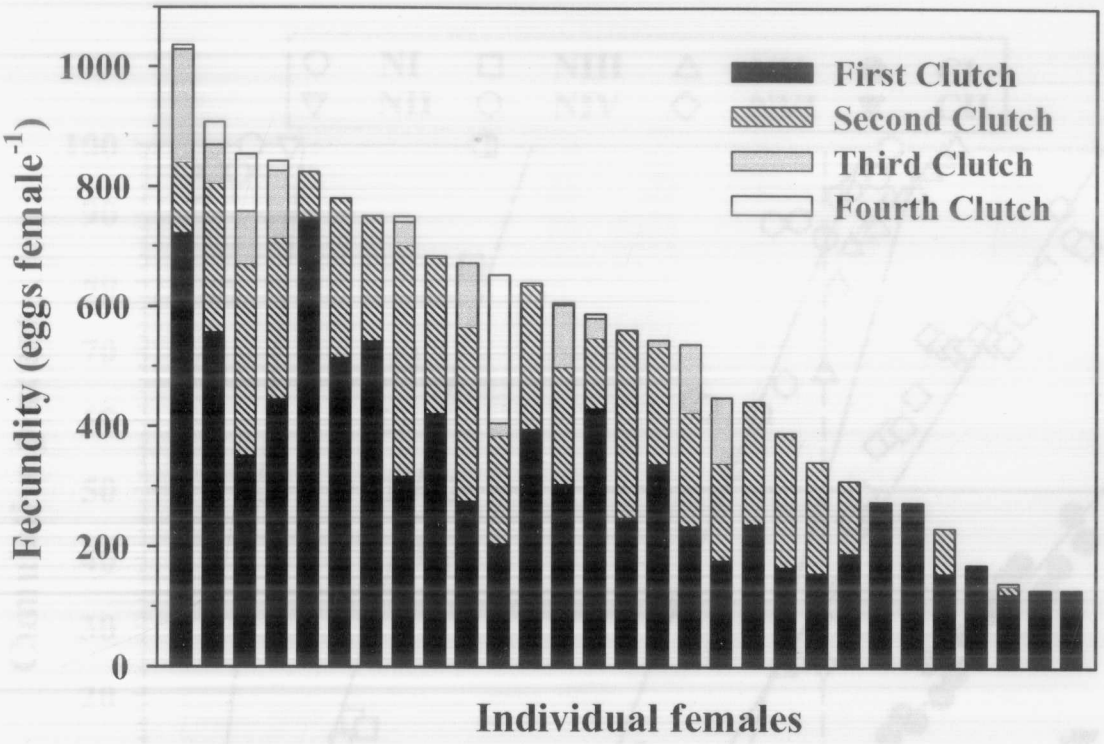

Figure 2.2 Fecundity of *Neocalanus flemingeri* females reared in the laboratory at 5°C. Some first clutches may contain more than one clutch.

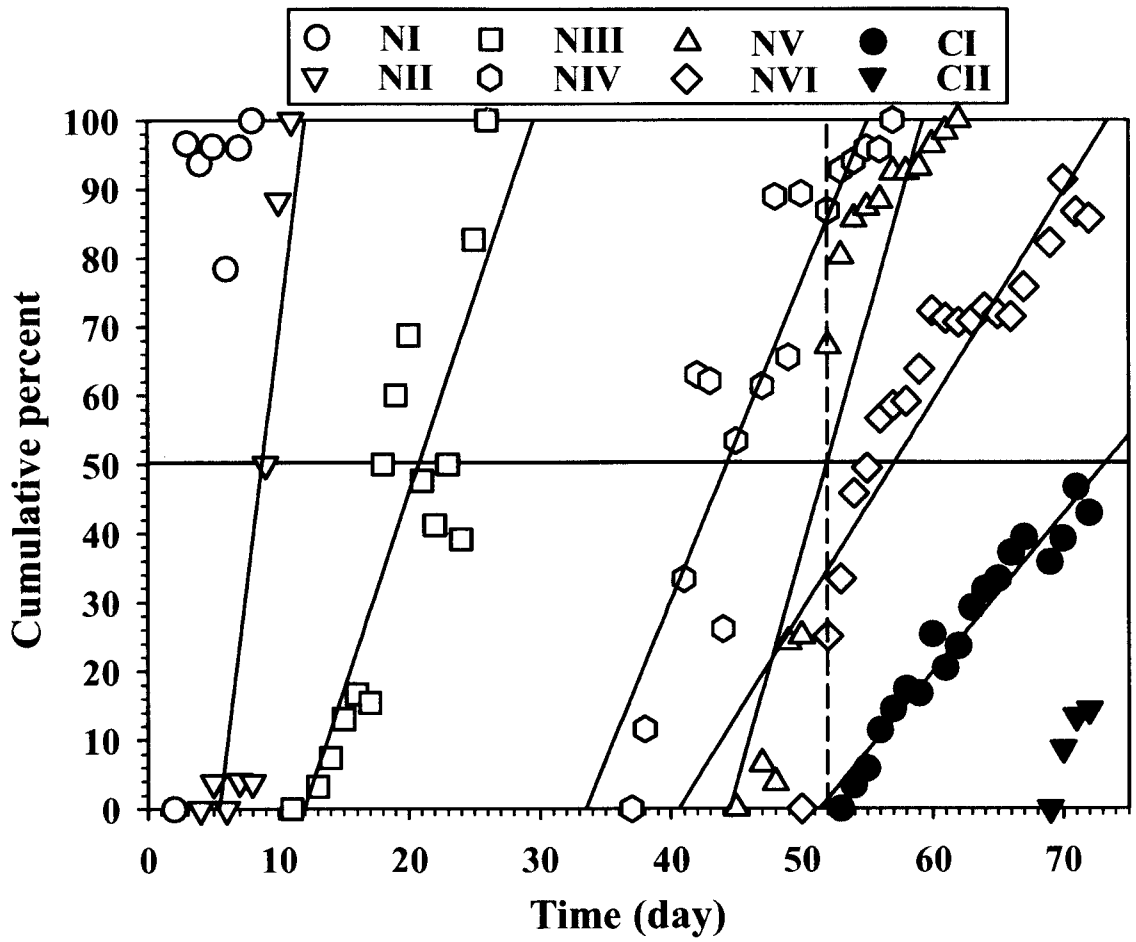

Figure 2.3 Daily cumulative percent at stage of *Neocalanus flemingeri* nauplii (open symbols) and early copepodite (closed symbols) stages reared in the laboratory at 5°C. Solid lines show statistically significant linear regressions ($P<0.01$). Dashed line indicates a change in methodology from population sub sample to non-destructive population census. n=17-31 for destructive sampling and n=14-256 for non-destructive sampling

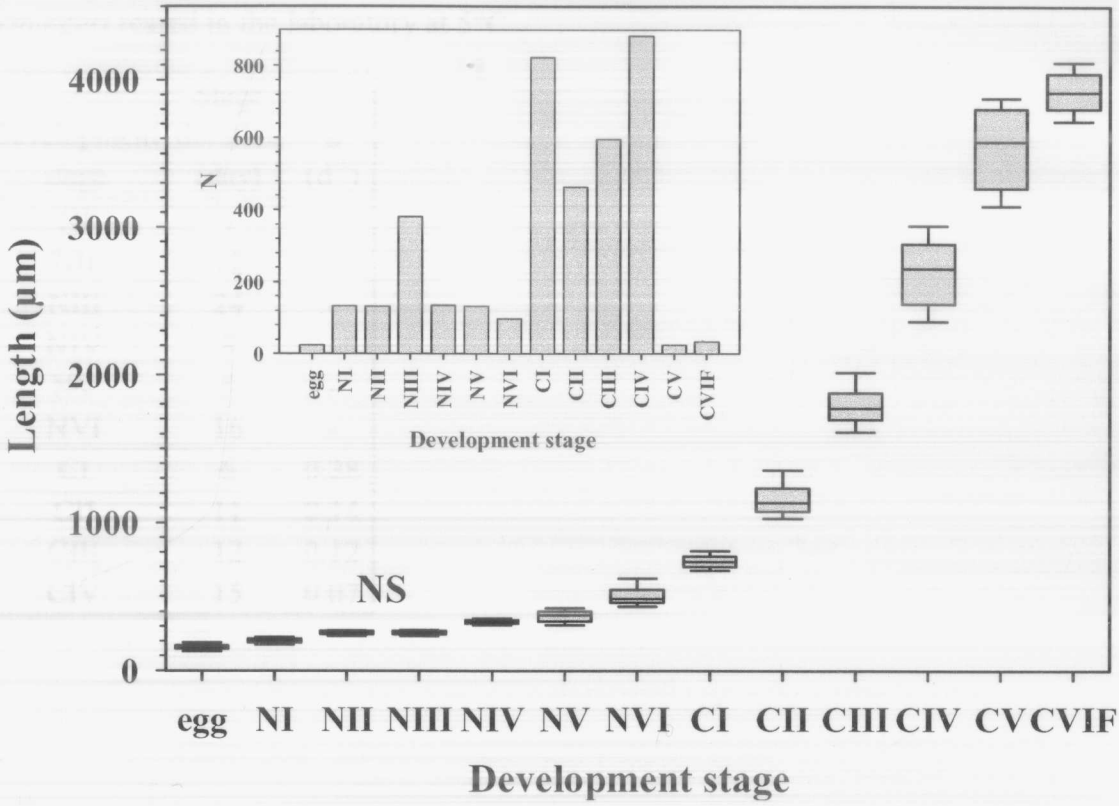

Figure 2.4 Naupliar total length or copepodite prosome length of *Neocalanus flemingeri* reared in the laboratory at 5°C. Boxes include from the 25th to the 75th percentiles, whiskers indicate the 10th and 90th percentiles and lines within each box display the median. Length increased significantly with stage ($P<0.001$) except between NII and NIII ($P=0.87$). Inset figure shows number of copepods measured at each development stage.

Table 2.1 Median stage durations and instantaneous growth rates (g) of *Neocalanus flemingeri* reared in the laboratory at 5°C.

Development stage	Stage duration (day)	g (d^{-1})
NI	7	-
NII	12	-
NIII	24	-
NIV	8	-
NV	5	-
NVI	16	-
CI	6	0.28
CII	11	0.14
CIII	12	0.12
CIV	15	0.07

CONCLUSION

This thesis provides the first account of the development of *N. flemingeri* from eggs to CV and copepodite growth rates determined in a laboratory. It contributes to the existing knowledge of *C. abdominalis* allowing predictions of development to be made over its apparent temperature range. Overall, this information helps clarify the life history patterns of these two species and allows production to be estimated and models of secondary production created for conditions within the Gulf of Alaska. It also allows us to compare rates with *in situ* populations, which shows that while *C. abdominalis* may be food limited in the field during summer and fall, *N. flemingeri* is not likely food limited during late spring.

Development of these two species is quite different with a generation of *Centropages abdominalis* complete in less than 6 weeks at 7°C vs *Neocalanus flemingeri*'s annual life cycle. Even ignoring the CV and adult durations, it took *N. flemingeri* almost two months longer to complete a generation at only 2°C lower temperature and at similar food conditions. Of course, they have adapted different life history methods, with *N. flemingeri* accumulating large lipid stores to fuel diapause stage, reproduction, and egg production (Mackas and Tsuda, 1999) while *C. abdominalis* is geared to take advantage of temporally limited conditions, such as upwelling events (Gomez-Gutierrez and Peterson, 1999), and production of diapause eggs when conditions become unfavorable (Liang *et al.*, 1994). From an ecological perspective, *C. abdominalis* could be characterized as possessing r-selected traits of rapid growth and development, temporally high abundances, and small egg investment (73 ± 4 μm (mean ±

S.D.) egg diameter). Conversely, *N. flemingeri* could be characterized as possessing k-selected traits of longer life span, consistent seasonal production cycles, larger size, and a greater egg investment (156 ± 4 µm (mean ± S.D.) egg diameter). Thus *N. flemingeri* is a more predictably consistent player in this system, while *C. abdominalis* varies much more temporally and spatially in its contribution to the ecosystem.

Average copepodite growth rates were similar between these species, averaging 0.17 d^{-1} at 7°C and 0.15 d^{-1} at 5°C for *C. abdominalis* and *N. flemingeri*, respectively, and decrease with stage. Since this growth was sustained over a longer duration for *N. flemingeri* which were nearly twice the size at CI, they attain a much larger female body size (nearly 3 ½ times) and more lipid reserves than *C. abdominalis*. Thus, even though growth rates are similar and imply an average doubling of body mass of about 5 days for both species, *N. flemingeri* contributes much more biomass to secondary production.

Determining life history traits of copepod species is an important requirement to understand their ecological function within marine systems. Knowledge of differences and similarities between these two species will lead to research to determine how they might affect food web interactions. Analyzing life history metrics at other temperatures will help determine how these species might respond to interannual variations and long term climate changes, which already appears to be moderating zooplankton biomass and *Neocalanus plumchrus* developmental timing in parts of the subarctic Pacific (Mackas and Tsuda, 1999). Establishing life history traits of other copepods important within the Gulf of Alaska such as *N. cristatus*, *N. plumchrus*, *Pseudocalanus* spp., *Metridia pacifica*, *Calanus* spp., *Acartia longeremis*, and *Oithona similis* will help round out our

understanding of ecosystem processes in this region as well as developing precise models of secondary production. Such efforts are currently in progress for several of these species by colleagues within the NEP GLOBEC program.

REFERENCES

Gomez-Gutierrez, J. and Peterson, W. T. (1999) Egg production rates of eight calanoid copepod species during summer 1997 off Newport, Oregon, USA. *J. Plankton Res.*, **21**, 637-657.

Liang, D., Uye, S. and Onbe, T. (1994) Production and loss of eggs in the calanoid copepod *Centropages abdominalis* Sato in Fukuyama Harbor, the Inland Sea of Japan. *Bull. Plankton Soc. Jap.*, **41**, 131-142.

Mackas, D. and Tsuda, A. (1999) Mesozooplankton in the eastern and western subarctic Pacific: community structure, seasonal life histories, and interannual variability. *Prog. Oceanogr.*, **43**, 335-363.

Saito, H. and Tsuda, A. (2000) Egg production and early development of the subarctic copepods *Neocalanus cristatus*, *N. plumchrus* and *N. flemingeri*. *Deep-Sea Res. I.*, **47**, 2141-2158.